

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
24 October 2002 (24.10.2002)

PCT

(10) International Publication Number  
**WO 02/082973 A2**

- (51) International Patent Classification<sup>7</sup>: **A61B**
- (21) International Application Number: **PCT/US02/11519**
- (22) International Filing Date: **15 April 2002 (15.04.2002)**
- (25) Filing Language: **English**
- (26) Publication Language: **English**
- (30) Priority Data:  
**60/284,107** **16 April 2001 (16.04.2001)** **US**
- (71) Applicant (for all designated States except US): **BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM [US/US]; 201 West Seventh Street, Austin, TX 78701 (US).**
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **WHANG, Kyumin [KR/US]; 14027 Cougar Rock Dr., San Antonio, TX 78230 (US). SATSANGI, Neera [US/US]; 12823 Hunting Bear, San Antonio, TX 78249 (US). NORLING, Barry, K. [US/US]; 13427 Shorecliff St., San Antonio, TX 78248-1213 (US).**
- (74) Agent: **SIBLEY, Kenneth, D.; Myers Bigel Sibley & Sajovec, Post Office Box 37428, Raleigh, NC 27627 (US).**
- (81) Designated States (national): **AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.**
- (84) Designated States (regional): **ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).**

**Published:**

— *without international search report and to be republished upon receipt of that report*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: **OSTEOTROPIC BIOMATERIALS, METHODS OF USE THEREOF AND IMPLANT SYSTEMS INCORPORATING THE SAME**

(57) Abstract: An osteotropic compound of the Formula P-L-S is described, wherein P is a biodegradable polymer such as a polyester polymer, L is a linking group such as a covalent bond or carbonyl, and S is an osteotropic group such as a statin. Scaffold bodies formed from such compounds are described, along with such bodies containing bone cells such as bone marrow cells and methods of use thereof.

**WO 02/082973 A2**

**BEST AVAILABLE COPY**

- 1 -

5

OSTEOTROPIC BIOMATERIALS, METHODS OF USE THEREOF  
AND IMPLANT SYSTEMS INCORPORATING THE SAME

10

Kyumin Whang, Neera Satsangi, and Barry K. Norling

Field of the Invention

The present invention concerns compounds, materials, systems and methods for stimulating the production of bone growth in a subject in need thereof.

15

Background of the Invention

Recombinant human bone morphogenetic protein-2 (BMP-2) has been produced to stimulate the growth of bone *in vivo*. In addition, the 3-hydroxy-3-methylglutaryl coenzyme A (HMG Co-A) reductase inhibitors (statins such as simvastatin, and lovastatin) have recently been shown to induce expression of the BMP-2 gene in bone cells to enhance bone formation. A problem with the application of these findings in the clinic, however, is the high cost of hBMP-2, along with the rapid dispersion of hBMP-2 and statins after administration. Thus, an appropriate delivery system would be extremely useful.

25

Mundy et al., *Stimulation of Bone Formation in Vitro and in Rodents by Statins*, Science 286, 1946-1949 (1999), describes the stimulation of bone formation by statins *in vitro* and *in vivo*. Particular delivery systems are neither suggested nor described.

30

Healy et al. *Methods of Fabricating Emulsion Freeze-Dried Scaffold Bodies and Resulting Products*, U.S. Patent No. 5,723,508, describes scaffold bodies, suitable for use as bone implants, formed by freeze-drying emulsions of polymer and water solutions. Suitable polymers include polymers of polyglycolic acid, polylactic acid,

- 2 -

and their copolymers (column 3, lines 27-32). It is suggested that water-soluble therapeutic agents such as bone morphogenetic proteins can be incorporated into the polymers (column 3, line 36-39 and Example IV (using bovine serum albumin)), but particular procedures for carrying this out are neither suggested nor disclosed.

5       **Gasper et al.**, *Compositions and Methods for Stimulating Bone Growth*, U.S. Patent No. 6,080,779, describes, among other things, a variety of different statin analogs that have activity in enhancing bone. A variety of different delivery techniques are described, and the patent states that "for local administration, the delivery vehicle preferably provides a matrix for the growing bone or cartilage, and  
10 more preferably is a vehicle that can be absorbed by the subject without adverse effects." (Column 6, lines 48-52).

**J. Jagur-Grodzinski**, *Biomedical application of functional polymers*, *Reactive & Functional Polymers* 39, 99-138 (1999), generally describes the use of "polymeric prodrugs" obtained "by conjugating biocompatible polymeric molecules  
15 with appropriate drugs." Use of absorbable polymers for bone repair is briefly mentioned in the paragraph bridging pages 111-112.

**J. Oh et al.**, *Controlled Drug Delivery System Using the Conjugation of Drug to Biodegradable Polyester*, WO 99/59548, describes covalent conjugates of molecules to be released with biodegradable polyester polymers. The polymers  
20 include poly (lactic acid), poly (glycolic acid), and copolymers thereof (page 8, lines 15-20). The conjugation linkage is described as being, among other things, an ester or amide bond (page 9, lines 3-7). The conjugation technique is described as being the coupling of the active molecule to a carboxyl or hydroxyl group in the polymer (page 14 line 25 to page 15 line 9). Drugs to be coupled are, in general, peptides or  
25 therapeutic agents (page 6, lines 4-21). The compositions are described as being formed into microspheres, nanoparticles or films (page 10, lines 13-15). Techniques for bone implantation and treatment are neither suggested nor described.

      In view of the foregoing, there remains a need for new ways to administer osteotropic compounds such as statins to subjects in need thereof, and particularly for  
30 ways to administer such compounds in a controlled manner effective to stimulate bone growth at the site of an injury or fracture.

- 3 -

### Summary of the Invention

A first aspect of the present invention is an osteoinductive protein synthesizing implant system. The system comprises a porous scaffold or support structure, the porous scaffold formed from (i) a biodegradable polymer in combination with (ii) a 3-hydroxy-3-methylglutaryl coenzyme A (HMG Co-A) reductase inhibitor. Bone cells (e.g., bone marrow cells) are impregnated in the porous scaffold, with the HMG Co-A reductase inhibitor included in the scaffold in an amount effective to stimulate the formation of bone tissue from the bone cells (e.g., when the scaffold is implanted in a subject as described below). Without wishing to be bound to any particular theory of the invention, it is believed that the HMG Co-A reductase inhibitor is included in the scaffold in an amount effective to stimulate the production of bone morphogenetic protein-2 (BMP-2) from the bone cells, which in turn stimulates the formation of bone tissue from the bone cells.

In a preferred embodiment of the foregoing implant system, the HMG Co-A reductase inhibitor is covalently coupled to the biodegradable polymer (as described in greater detail below). Such covalent coupling of the HMG Co-A reductase inhibitor to the biodegradable polymer advantageously serves to control the release of the HMG Co-A reductase inhibitor to the bone cells within the scaffold.

A second aspect of the present invention is a method of stimulating bone growth in a subject in need of such treatment, comprising implanting an implant system as described above into the subject (e.g., in, on or adjacent a broken bone or a bone or region requiring the growth of bone tissue).

A third aspect of the present invention is an implantable porous scaffold formed from an osteotropic compound of the Formula:

25

P-L-S

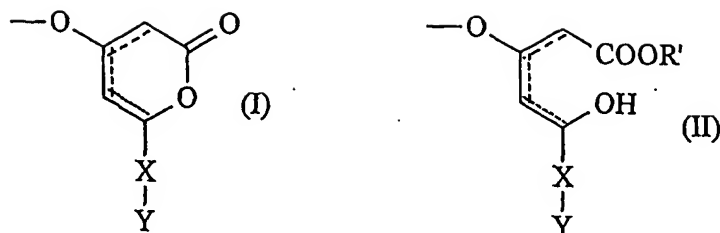
wherein:

30 P is a biodegradable polymer (preferably a polyester polymer);

L is a linking group; and

S is an osteotropic group, and preferably an osteotropic group according to Formula I or Formula II:

- 4 -



wherein X in each of formulas (I) and (II) represents an alkylene, alkenylene, or alkynylene linker; Y represents one or more carbocyclic or heterocyclic rings wherein, when Y comprises two or more rings, the rings may be fused; R' represents a cation, H or a substituted or unsubstituted alkyl group; and the dotted lines represent optional  $\pi$  bonds.

A still further aspect of the present invention is an osteotropic compound of the Formula:

P-L-S

wherein:

P is a biodegradable polymer (preferably a polyester polymer such as poly(lactic acid), poly(glycolic acid), or copolymer thereof);

L is a linking group; and

S is an osteotropic group (preferably as described above).

The foregoing and other objects and aspects of the present invention are explained in greater detail in the drawings herein and the specification set forth below.

### Brief Description of the Drawings

Figure 1 shows a simplified schematic diagram of an illustrative osteogenic-poly(lactide) (PLG-STAT) synthesis.

Figure 2 shows a UV spectra of simvastatin (Sim) dissolved in methanol (MeOH), simvastatin in methylene chloride (MC), simvastatin in Dulbecco's Modified Eagle's Media (DMEM), simvastatin and PLG in MC and Osteogenic-PLG (OG-PLG) in MC. All have simvastatin concentrations of 50  $\mu\text{g/mL}$ .

Figure 3 shows a concentration curve for simvastatin in MeOH.

- 5 -

Figure 4 shows *in vitro* release curves of PLG + Simvastatin (4 mg/g PLG) and OG-PLG (100% w/w PLG). Note the quicker initial release and the more gradual continued release by OG-PLG.

Figure 5 shows an Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) spectrum of OG-PLG. Peaks to note are at 2958, 2919, 2851, and 1022  $\text{cm}^{-1}$ .

Figure 6 shows H&E stained sections of clavicular cross-sectioned bone. Large arrow point to the position of films and the small arrows point to areas of new bone formation.

#### Detailed Description of the Preferred Embodiments

The present invention is intended for use on human subjects or patients, but may also be used on animal subjects (particularly dogs, cats, pigs, and other mammalian species) for veterinary purposes or for the purpose of medical product screening and development.

The disclosures of all United States patent references cited herein are to be incorporated herein by reference in their entirety.

**Biodegradable polymers.** Biodegradable polymers (e.g., biocompatible and bioabsorbable polymers) that may be used to carry out the present invention include, but are not limited to, poly(hydroxybutyrate), polycarbonates, polyacrylates, polyanhydrides, poly(ortho esters), poly(phosphoesters), polyesters, polyamides (such as polyamides derived from D-glucose), polyphosphazenes, poly(*p*-dioxane), poly(amino acid), polyglactin, and copolymers thereof, erodable hydrogels, natural polymers such as collagen and chitosan, etc.. See, e.g., U.S. Patent No. 5,723,508 to Healy et al. Particular examples of suitable biodegradable polymers include, but are not limited to, aliphatic polyester polymers such as poly(lactic acid), poly(glycolic acid), poly(D-lactic-co-glycolic acid), poly(L-lactic-co-glycolic acid), poly (D,L-lactic-co-glycolic acid), poly(caprolactone), poly(valerolactone), poly(hydroxy butyrate) (including poly(hydroxy butyrate valerate)), poly(hydrovalerate), polydioxanone, poly(propylene fumarate), etc., including copolymers thereof such as polylactic acid-polyethylene glycol block copolymer, and poly(ethyleneoxide)-poly(butylene tetraphthalate), poly(lactic acid-co-lysine), etc.. See, e.g., J. Oh et al.,

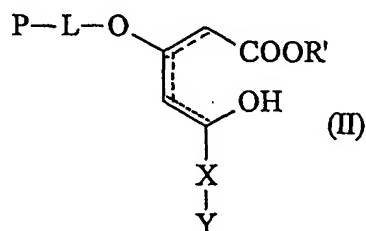
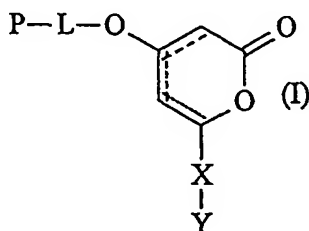
- 6 -

PCT Application WO 99/59548 at page 2. Additional examples of biodegradable polymers are set forth in U.S. Patent No. 5,916,585 to Cook et al. at col. 9 line 53 to col. 10 line 22. The molecular weight (that is, average molecular weight) of the polymer may be from 1,000, 10,000, 100,000 or 500,000 to 2,000,000 or 4,000,000 Daltons, or more. Currently, average molecular weights of about 1,000,000 Daltons are preferred.

**HMG Co-A reductase inhibitors.** Any suitable HMG Co-A reductase inhibitor may be used to carry out the present invention (*e.g.*, to form osteotropic groups on the compounds herein). Numerous statins that are HMG Co-A reductase inhibitors are known; examples are disclosed, for example, in U.S. Patent No. 6,022,887 to Gasper et al., and in B. Roth et al., *J. Med. Chem.* 34, 463-466 (1991). Examples of a variety of statins that may be used to carry out the present invention are provided in conjunction with the description of polymers below (where hydroxyl groups are removed from the statins and replaced with bonds to the linking groups).

Specific examples of statins that may be used to carry out the present invention include, but are not limited to, atorvastatin (Merck Index monograph number 897; 12<sup>th</sup> Edition 1996), fluvastatin, (Merck Index monograph number 4250), lovastatin (Merck Index monograph number 5616), mevastatin (Merck Index monograph number 6251), pravastatin (Merck Index monograph number 7894 for pravastatin sodium), and simvastatin (Merck Index monograph number 8686).

In general, compounds of the invention formed with statins as the osteotropic group or HMG Co-A reductase inhibitor are those in which S is an osteotropic group according to Formula I or Formula II:



25

wherein:

- 7 -

X in each of formulas (I) and (II) represents an unsubstituted alkylene, alkenylene, or alkynylene linker of 2-6 C;

Y represents one or more carbocyclic or heterocyclic rings wherein, when Y comprises two or more rings, said rings may be fused; and

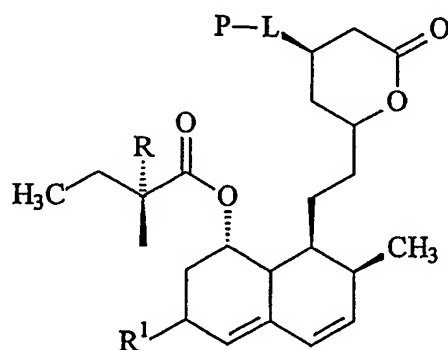
5 R' represents a cation, H or a substituted or unsubstituted alkyl group of 1-6 C;

the dotted lines represent optional  $\pi$  bonds;

P is a polymer as described herein; and

L is a linking group as described herein.

10 In particular examples of the foregoing where S is based upon mevastatin, lovastatin or simvastatin, S is an osteotropic group of the formula:



15

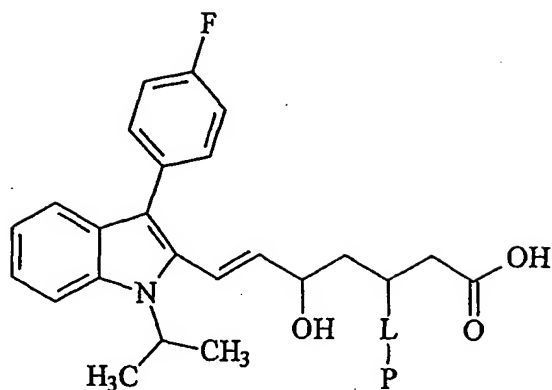
where R is H or CH<sub>3</sub> and R<sup>1</sup> is H or CH<sub>3</sub> (for mevastatin R and R<sup>1</sup> are H, for lovastatin R is H and R<sup>1</sup> is CH<sub>3</sub>, and for simvastatin R is H and R<sup>1</sup> is CH<sub>3</sub>).

In further examples of the foregoing where S is based upon fluvastatin, S is an osteotropic group of the formula:

20

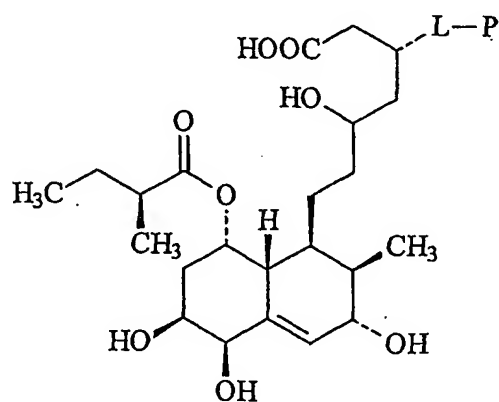


- 8 -



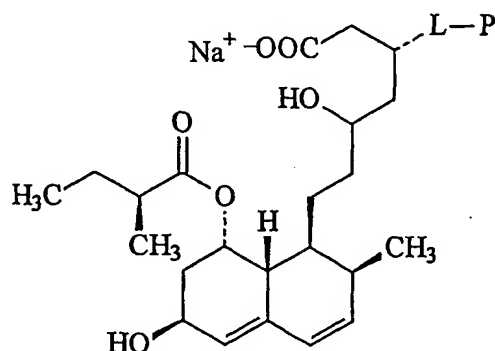
In further examples of the foregoing where S is based upon pravastatin, S is an osteotropic group of the formula:

5



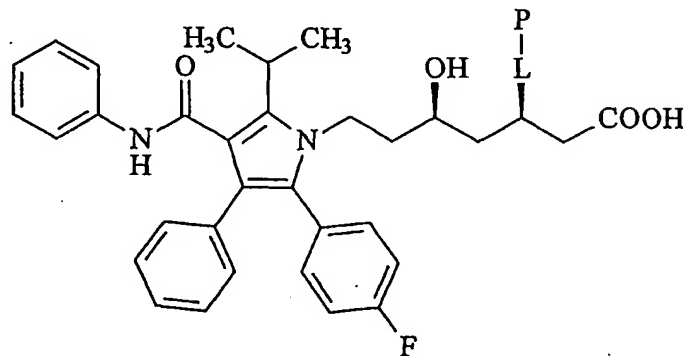
- 9 -

In further examples of the foregoing where S is based upon pravastatin sodium, S is an osteotropic group of the formula:



5

In further examples of the foregoing where S is based upon atorvastatin, S is an osteotropic group of the formula:



10

Numerous other particular osteotropic groups S will be apparent to those skilled in the art based upon the examples given above.

**Linking groups.** Any suitable linking group may be used to link the osteotropic group to the polymer to carry out the present invention. Depending upon the particular chemistry employed, the linking group may represent a linkage by an ester bond, amide bond, anhydride bond, carbonate bond, urea bond, urethane bond, thioester bond, disulfide bond, imine bond, carbonate bond, etc. The linking group

15

- 10 -

may be a direct covalent bond between the polymer and the osteotropic group. A spacer group such as a C1-C6 alkylene group may be included in the linking group. See, e.g., J. Oh et al., PCT Application WO99/59548 at page 15.

Suitable linking groups are also described in U.S. Patent No. 6,017,940 to Petrie et al. Thus the linking group, L, may be a covalent bond or any group having a valence of at least two and covering a linear distance of from about 1.5 to about 15 Angstroms, including those that contain cyclic moieties, that meet this spatial requirement. Useful linkers include, for example, flexible non-conjugating linkers and flexible conjugating linkers. Flexible non-conjugating linkers are those that link only one position of the polymer to one position of the osteotropic group, and provide only a single covalent bond or a single chain therebetween. The chain may contain branches or cyclic portions in the chain. The linker atoms in the chain itself rotate freely around single covalent bonds, and thus the linker has more than two degrees of freedom. Example non-conjugating linkers, besides a covalent bond, are those of the formulae:  $\text{—NR—}$ ,  $\text{—CR}_2\text{—}$ ,  $\text{—S—}$ , or  $\text{—O—}$ , wherein R is H or alkyl (1-6C), more preferably H or lower alkyl (1-4C) and more preferably H. Also preferred are those of the formulae:  $\text{—NRCO—}$ ,  $\text{—CONR—}$ ,  $\text{—CR}_2\text{S—}$ ,  $\text{—SCR}_2\text{—}$ ,  $\text{—OCR}_2\text{—}$ ,  $\text{—CR}_2\text{O—}$ ,  $\text{—NRNR—}$ ,  $\text{—CR}_2\text{CR}_2\text{—}$ ,  $\text{—NRSO}_2\text{—}$ ,  $\text{—SO}_2\text{NR—}$ ,  $\text{—CR}_2\text{CO—}$ ,  $\text{—COCR}_2\text{—}$ , and  $\text{—NR—NR—CO—CR}_2\text{—}$  and its complement  $\text{—CR}_2\text{—CO—NR—NR—}$ , including the isosteres thereof. Also preferred are those of the formulae:  $\text{—NR(CR}_2)_2\text{NR—}$ ,  $\text{—O(CR}_2)_2\text{O—}$ , and  $\text{—S(CR}_2)_2\text{S—}$ , including the isosteres thereof. Example flexible conjugating linkers are those that link only one position of the polymer to one position of the osteotropic group, but incorporate at least one double or triple bond or one or more cyclic systems and thus have only two degrees of freedom. A flexible conjugating linker may form a completely conjugated  $\pi$ -bond linking system between S and P, thus providing for co-planarity of S and P. Examples of useful flexible conjugating linkers include:  $\text{—RC=CR—}$ ;  $\text{—N=N—}$ ;  $\text{—C}\equiv\text{C—}$ ;  $\text{—RC=N—}$ ;  $\text{—N=CR—}$ ;  $\text{—NR—N=CR—}$ ;  $\text{—NR—NR—CO—CR=CR—}$ ; and the like, where R is H or alkyl (1-6C); preferably H or lower alkyl (1-4C); and more preferably H.

- 11 -

**Scaffold body production and use.** Scaffold bodies may be formed from polymers as described above, where the polymers are either covalently linked to an osteotropic group such as a statin as described above, or where the scaffold bodies are simply impregnated with osteotropic compounds such as statins as described above, in accordance with known techniques. Preferably the scaffold bodies are formed from polymers covalently linked to an osteotropic group, as described above. In a preferred embodiment, the scaffold bodies are formed by emulsion freeze dry casting, such as described in U.S. Patent No. 5,723,508 to Healy et al. In general, such techniques involve preparing an emulsion with a polar solvent dispersed in a non-polar solvent which is immiscible with the polar solvent, the non-polar solvent having dissolved therein a polymer as described above. The polymer preferably has an osteotropic group covalently coupled thereto as described above, or in the alternative the osteotropic group may be provided in a free form included within the solvent. The emulsion is then frozen in a mold under conditions which convert both of the solvents to solids, preferably without breaking the emulsion or throwing the polymer out of solution, to thereby obtain a solid frozen body of molded shape. The resulting body is then freeze-dried to partially or completely convert the body to a porous solid. The process can then optionally be repeated until the body is entirely a porous open-celled body. Once completed the body may be sterilized and packaged in a sterile aspect package or container for subsequent use, in accordance with standard techniques.

The scaffold body described above may be used in a variety of ways, such as described in U.S. Patent No. 5,723,508 to Healy et al. Preferably, the body is impregnated with bone cells, prior to implantation. The bone cells may in general be bone marrow cells, and more particularly may be osteoblasts, stem cells, progenitor cells, or combinations or mixtures thereof. The bone cells may be autologous or non-autologous cells, but in a preferred embodiment the bone cells are withdrawn from the subject into which the implant system is to be implanted prior to implantation (e.g., immediately prior to implantation, or withdrawn and stored, processed, etc. for subsequent use). The bone cells (in a physiologically acceptable media such as sterile saline solution) are then inserted into the scaffold by any suitable technique, such as pumping, use of vacuum systems, soaking, absorption, injection (e.g., by injection into multiple points therein with a syringe), or combinations thereof. In general, from

- 12 -

1,000, 2,000, 5,000 or 10,000 to 100,000 or even 100 million cells per cubic centimeter of media (which media is carried by or contained in the scaffold to be implanted). Once the cells are introduced into the scaffold, the scaffold may then be implanted into the subject. The scaffold body may be used in a preformed shape, or may be cut to the appropriate size at the time of use. Such scaffold implants may be used for any of a variety of different procedures, including but not limited to the repair of non union defects, treatment of osteonecrosis, repair of bone cysts, bone regeneration in large bone defects, periodontal bone regeneration, sinus lifts, alveolar ridge augmentation, acute fracture healing, etc.

The present invention is explained in greater detail in the following non-limiting Examples.

#### EXAMPLE 1

##### Cell Delivery of Emulsion Freeze-Dried Scaffolds

Emulsion freeze-dried (EFD) scaffolds with 30- $\mu$ m median pore size were seeded with  $10^4$  fetal rat calvarial cells in 24-well plates by injection into the scaffold at six evenly distributed sites around the periphery and also in the center of the scaffold, and the scaffolds then cultured *in vitro*. The capillary pressure generated by pores less than 50  $\mu$ m absorbed the cell suspension, and the high porosity allowed even distribution of the suspension. After 7 days, final cell density was significantly higher in the scaffolds as compared to tissue culture polystyrene ( $p < 0.005$ ) as measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Alkaline phosphatase (ALP) assay indicated that ALP released from the cultures was similar.

In a separate study,  $10^5$  chondrocytes harvested from cow hooves were seeded onto EFD scaffolds (1.27 cm<sup>2</sup> x 0.5cm thick) transplanted into nude mice ectopic sites. After 1 month, histology stained with H&E and Alcian Blue revealed that the cells proliferated to fill the entire scaffold and that cartilage matrix was being produced by one month. This data indicates that EFD scaffolds can be used for cell transplantation.

## EXAMPLE 2

### In Vitro and In Vivo Delivery of Statins

EFD scaffolds with a median pore size of 145  $\mu\text{m}$  were loaded with lovastatin by dissolving into the organic phase with the poly(lactide-co-glycolide) (PLG) before  
5 homogenization. Efficiency of lovastatin loading was determined by dissolving the scaffold in methylene chloride and extracting lovastatin with methanol. High-pressure liquid chromatography (HPLC) showed over 90% recovery from scaffolds, and a HMG Co-A reductase assay showed full activity of the recovered lovastatin. Scaffolds were immersed in culture media for eight days to determine release kinetics. HPLC  
10 showed that the kinetics of lovastatin release was zero-order, with about 2.5  $\mu\text{g/day}$  being released from 30-mg scaffolds loaded with 64 or 222  $\mu\text{g}$  of lovastatin. Scaffolds, cut into 12 to 18 mg implants, loaded with four different concentrations of lovastatin (0, 0.25, 2.5 and 25 mg/g scaffold) were implanted subcutaneously *in vivo* over the calvaria of two-month old ICR Swiss mice. Constant delivery of small  
15 amount of lovastatin (4-40  $\mu\text{g}$  total amount) caused massive amount of new bone formation as compared to local subcutaneous injection of lovastatin (5 mg/kg/day, daily for 5 days, total of 625  $\mu\text{g}$ ). The area of new bone induced by lovastatin derived from scaffolds was two- to four-fold higher than local injection while the total amounts released from scaffolds were 15- to 30-fold lower (Whang et al. (2000)  
20 American Society for Bone and Mineral Research 22<sup>nd</sup> Annual Meeting, S225, Abstract F380). However, no bone was found on or inside the scaffolds, but rather only on the surface of the calvaria. It is believed that cells were not attracted to migrate into the scaffolds because lovastatin is not chemotactic and worked only when released to the surrounding where there were bone cells to be stimulated. This is  
25 probably why bone formation was seen inside the scaffolds with rhBMP-2 previously and not here (Whang et al. (1998) *J. Biomed. Mat. Res.* 42:491-499).

## EXAMPLE 3

### The Osteoinductive Protein Synthesizing Implant System (OPSIS)

30 An osteoinductive protein synthesizing implant system (OPSIS) has been developed to further amplify bone formation by making better use of the scaffold as a substrate. Bone marrow cells are seeded into scaffolds made with an osteotropic

biomaterial, Osteogenic-PLG (OG-PLG). The OPSIS may enhance bone formation via three methods. First, bone marrow cells will be induced to produce BMP-2 and attract cells to migrate into the scaffolds to form bone; second, the bone marrow cells will themselves be induced to form bone; and third, the scaffold microarchitecture will further enhance bone formation.

The osteotropic biomaterial, OG-PLG, is produced by grafting statins to the end of PLG. Figure 1 shows a schematic of the synthesis. The terminal carboxyl group of the polymer, whether glycolide or lactide, was activated as its acid chloride with oxalyl chloride, which was then esterified with the alcoholic functionality in simvastatin (Cal Biochem Corp., San Diego, CA). Specifically, PLG (2.64 g, 85:15 molar ratio, inherent viscosity 0.78 dL/g, Birmingham Polymers, Birmingham, AL) was dissolved in methylene chloride (MC, 10 mL, Aldrich, Milwaukee, WI) and oxalyl chloride (3 mL added slowly, Aldrich, Milwaukee, WI) and stirred at room temperature for 24 hours. Then, the solvent and excess oxalyl chloride was distilled off on a rotary evaporator at room temperature. The acid chloride of PLG was again dissolved in MC (10 mL) and stirred overnight with an equimolar amount of simvastatin (8.36 mg in 2 mL of MC) in the presence of anhydrous pyridine (3-4 drops, Aldrich, Milwaukee, WI) as a catalyst. The solvents were distilled off at reduced pressure and room temperature. Finally, the reaction mixture was washed three times with methanol (5 mL, Aldrich, Milwaukee, WI). This synthesis was also performed with lovastatin.

To help characterize OG-PLG and also measure release of OG-PLG fragments, the use of UV/Vis spectroscopy was further developed. Based on published reports (Vyas et al. (1990) *Drug Metabolism & Disposition* 18:203-211; Wang and Asgharnejad (2000) *J. Pharm. & Biomed. Anal.* 21:1243-1248), the presence of three distinct peaks of simvastatin dissolved in methanol (MeOH) was confirmed at 231, 240 and 248 nm (Figure 2). The UV spectrum of simvastatin dissolved in MC with and without PLG, with and without media, and at different simvastatin concentrations was analyzed. Due to the absorbance of MC at short wavelengths, there was scattering in the data below 230 nm. However, the three peaks were visible and usable for detecting simvastatin after subtraction of MC (Figure 2). The linear detectable concentration range of 1 to 50 µg/ml was not affected (Figure

3). When, PLG was co-dissolved with simvastatin in MC, PLG absorbed light at short wavelengths and so even more scatter was observed. However, a distinct peak at 248 nm was preserved and the use of this wavelength to determine simvastatin concentration was not affected (Figure 2). Finally, Dulbecco's Modified Eagle's Media (DMEM) also absorbed light at short wavelengths but only below 235 nm (Figure 2). Thus, the peak at 248 nm was not affected and could be used to determine simvastatin concentration (Whang et al. (2001) *Trans. Soc. Biomater.* In press). DMEM was used in the following studies because the *in vitro* release study was performed in media, a more accurate representation of what the cells would be seeing in *in vitro* cell studies.

The OG-PLG was further characterized using contact angle measurements, Attenuated Total Reflectance Fourier Transform Infrared spectroscopy (ATR-FTIR; Southwest Research Institute (San Antonio, TX)), and UV/Vis. PLG, PLG and simvastatin (PLG+Sim, 4 mg/g PLG) and OG-PLG were dissolved into MC at 20% w/v and cast onto glass slides to form films. Simvastatin concentration of 4 mg/g PLG was used because that was equivalent to the theoretical concentration of simvastatin in OG-PLG (i.e. 1 mole simvastatin to 1 mole PLG of  $\eta_{inh} = 0.78$  dL/g). Contact angles were found to be  $80^\circ \pm 1^\circ$ ,  $79^\circ \pm 1^\circ$  and  $97^\circ \pm 1^\circ$  for PLG, PLG+Sim and OG-PLG, respectively. The contact angle of OG-PLG was significantly greater than PLG or PLG+Sim ( $p < 0.05$ ). It was believed that this increase reflected the disappearance of the -OH group from either the simvastatin or the end of the PLG polymer from the reaction. The contact angle of the PLG+Sim was not different from PLG probably because simvastatin is more hydrophobic and so when exposed to air, it did not present itself on the surface. This was confirmed via the ATR-FTIR data presented below (Figure 5) (Whang et al. (2001) *Trans. Soc. Biomater.* In press).

ATR-FTIR spectroscopy revealed a change in the spectrum between PLG, PLG+Sim and OG-PLG. There were three distinct peaks at 2958, 2919 and 2851  $\text{cm}^{-1}$  for stock simvastatin powder (Figure 5). Neither PLG nor PLG+Sim spectra contained those peaks but OG-PLG contained them, though they were weak peaks (Figure 5). These peaks represent the -CH<sub>2</sub> and -CH<sub>3</sub> groups on simvastatin. Another notable peak was one at 1022  $\text{cm}^{-1}$  representing an ester group that did not exist in the other spectra. It is believed that this peak represents the ester group that was formed



- 16 -

during the reaction and either the simvastatin or the PLG lost an -OH group. Note that PLG+Sim did not demonstrate any of the three distinct peaks, leading one to believe that there was no simvastatin at the surface since ATR-FTIR detects only the material's surface chemistry (Whang et al. (2001) *Trans. Soc. Biomater.* In press).

5 UV/Vis on a solution of OG-PLG in MC did not show a quick characteristic drop in absorbance after the 248 nm peak but continued to decrease slowly past 300 nm (Figure 2). This further helps support that there is a difference in chemistry between PLG+Sim and OG-PLG. The peak at 248 nm was not affected and showed the same sensitivity to simvastatin concentration despite being attached to PLG.

10

#### EXAMPLE 4

##### In Vitro Controlled Release of Simvastatin

Films of PLG, PLG+Sim and OG-PLG were cast into scintillation vials and an *in vitro* release study in DMEM was conducted. The release was measured using  
15 UV/V is at 248 nm. Figure 4 shows the cumulative release curves of simvastatin from PLG+Sim, and OG-PLG fragments from OG-PLG. It appears that there was no simvastatin release from PLG+Sim until almost 20 days. This was probably because the simvastatin in PLG+Sim was not showing at the surface. The difference in the results with the previous study using scaffolds that showed a zero-ordered release can  
20 be reconciled by the fact that these films have very low specific surface area compared to the highly porous scaffolds. Conversely, the OG-PLG initially released quicker due to either molecules on the surface leaching out first, and/or simvastatin attached to low-molecular weight PLG leaching out without the need to degrade first. However, the subsequent release seems to be more gradual as compared to the sudden  
25 quick release of the simvastatin from PLG+Sim once it is allowed to diffuse out.

In short, the studies presented herein show that these scaffolds can be used to deliver cells for transplantation and statins with enhanced efficacy. However, with the diffusion-controlled statin delivery, new bone was found only on the calvaria and not in the scaffold because the statins are not chemotactic. Thus, a novel concept of an  
30 osteoinductive protein synthesizing implant system (OPSIS) consisting of a novel osteotropic biomaterial, OG-PLG, fabricated into a 3-D scaffold and seeded with bone marrow cells has been developed to further enhance bone regeneration. These studies

- 17 -

confirmed the synthesis of OG-PLG. An UV-Vis assay was developed to measure *in vitro* release and have shown that degradation-controlled release allows for a quicker initial release with a more gradual prolonged release as compared to diffusion-controlled release. It is expected that the OPSIS would continually synthesize *in situ* BMP-2 by stimulating seeded bone marrow cells via the OG-PLG. It is also expected that the scaffold microarchitecture would further enhance the mass and speed of new bone formation. Clinically, the clinician can cut the scaffold into the appropriate shape and size, draw the patient's bone marrow, inject it into the scaffold, and implant the OPSIS into the patient.

10

### EXAMPLE 5

#### In Vivo Efficacy of OG-PLG

Sterile films made with PLG (Negative Control; 85:15 molar ratio,  $\eta_{inh} = 0.78$  dL/g Birmingham Polymers, Birmingham, AL) or OG-PLG were fabricated by solvent casting using methylene chloride (MC) as solvent (20% w/v) onto a sterile glass petri dish and dried 24 hours in a sterile hood and under Class 1000 clean room conditions. The films were then exposed to a very strong vacuum (5 mTorr) for 48 hours to rid of any residual solvent. These films were cut into 1-cm<sup>2</sup> films, weighed and then implanted subcutaneously on top of adult inbred Lewis rat calvaria (6 months old). After 8 weeks, rats were euthanized, and implants and surrounding tissue excised to assess the amount and rate of new bone formation via histology and histomorphometry on H&E, Goldner Trichrome and Tetracycline-labeled sections.

Figure 6 shows that most of the new bone was formed near the suture lines next to the edge of the films along with periodic pockets of new bone below the films. Note that there is bone formation in negative controls (PLG) in this model. Histomorphometry suggests that there is more new bone in the OG-PLG group than negative controls ( $19038 \pm 7803$  vs.  $14624 \pm 5568$  pixels of new bone area) suggesting that the released OG-PLG is osteotropic.

The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

30

- 18 -

**THAT WHICH IS CLAIMED IS:**

1. An osteoinductive protein synthesizing implant system comprising:  
a porous scaffold, said porous scaffold formed from (i) a biodegradable polymer in combination with (ii) a 3-hydroxy-3-methylglutaryl coenzyme A (HMG  
5 Co-A) reductase inhibitor; and  
bone cells impregnated in said porous scaffold, with said HMG Co-A reductase inhibitor included in said scaffold in an amount effective to stimulate the formation of bone tissue from said bone cells.
- 10 2. The implant according to claim 1, wherein said HMG Co-A reductase inhibitor is included in said scaffold in an amount effective to stimulate the production of bone morphogenetic protein-2 (BMP-2) from said bone marrow cells.
3. The implant according to claim 1, wherein said bone cells are bone marrow  
15 cells.
4. The implant according to claim 1, wherein said bone cells are osteoblasts, stem cells or progenitor cells.
- 20 5. The implant according to claim 1, wherein said biodegradable polymer is selected from the group consisting of poly alpha-hydroxy acids, polyanhydrides, polyorthoesters, polyacrylates, polycaprolactones, polycarbonates, polyfumarates, and copolymers thereof.
- 25 6. The implant according to claim 1, wherein said biodegradable polymer is a polyester polymer selected from the group consisting of poly(lactic acid), poly(glycolic acid), poly(caprolactone), and copolymers thereof.
- 30 7. The implant according to claim 1, wherein said scaffold is produced by the process of emulsion- freeze-dry casting.

- 19 -

8. A method of stimulating bone growth in a subject in need of such treatment, comprising:

implanting an implant system according to claim 1 into said subject.

5 9. The method according to claim 8, wherein said bone cells are autologous or non-autologous cells.

10 10. A method according to claim 8, wherein said bone cells are withdrawn from said subject in which said implant system is to be implanted and then inserted into said scaffold prior to said implanting step.

11. An implantable porous scaffold formed from an osteotropic compound of the Formula:

P-L-S

15

wherein:

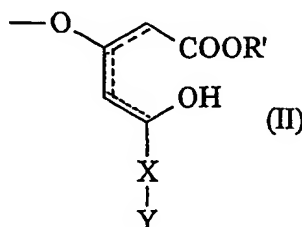
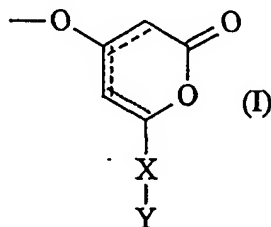
P is a biodegradable polymer;

L is a linking group; and

S is an osteotropic group.

20

12. The scaffold according to claim 11, wherein S is an osteotropic group according to Formula I or Formula II:



wherein:

25 X in each of formulas (I) and (II) represents an unsubstituted alkylene, alkenylene, or alkynylene linker of 2-6 C;

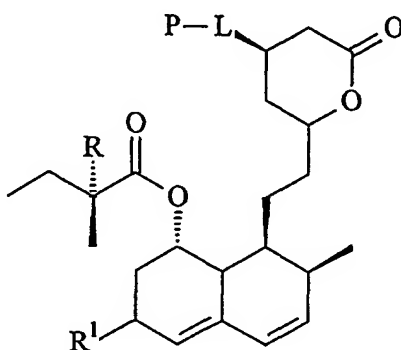
- 20 -

Y represents one or more carbocyclic or heterocyclic rings wherein, when Y comprises two or more rings, said rings may be fused; and

R' represents a cation, H or a substituted or unsubstituted alkyl group of 1-6 C; and

5 the dotted lines represent optional  $\pi$  bonds.

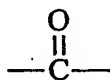
13. The scaffold according to claim 11, wherein S is an osteotropic group of the formula:



10

where R is H or CH<sub>3</sub> and R<sup>1</sup> is H or CH<sub>3</sub>.

14. The scaffold according to claim 11, wherein said linking group is a carbonyl group of the formula:



15

wherein said carbonyl group represents an end-terminal carboxyl group of said polymer to which S is covalently joined by an ester linkage.

15. The scaffold according to claim 11, wherein said biodegradable polymer  
20 is selected from the group consisting of poly alpha-hydroxy acids, polyanhydrides, polyorthoesters, polyacrylates, polycaprolactones, polycarbonates, polyfumarates, and copolymers thereof.

- 21 -

16. The scaffold according to claim 11, wherein said biodegradable polymer is a polyester polymer selected from the group consisting of poly(lactic acid), poly(glycolic acid), poly(caprolactone), and copolymers thereof.

5

17. An osteotropic compound of the Formula:

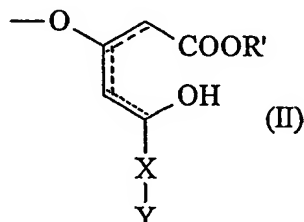
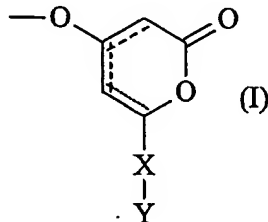
P-L-S

wherein:

- 10 P is a biodegradable polymer;  
L is a linking group; and  
S is an osteotropic group.

18. The compound according to claim 17, wherein S is an osteotropic group according to Formula I or Formula II:

15



wherein:

X in each of formulas (I) and (II) represents an unsubstituted alkylene, alkenylene, or alkynylene linker of 2-6 C;

20 Y represents one or more carbocyclic or heterocyclic rings wherein, when Y comprises two or more rings, said rings may be fused; and

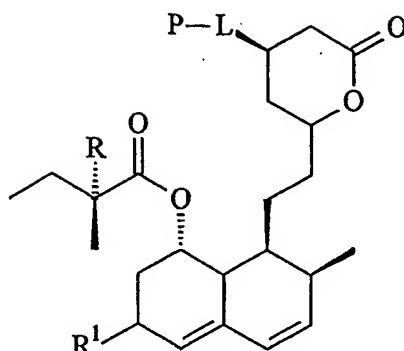
R' represents a cation, H or a substituted or unsubstituted alkyl group of 1-6 C; and

the dotted lines represent optional  $\pi$  bonds.

25

19. The compound according to claim 17, wherein S is an osteotropic group of the formula:

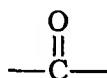
- 22 -



where R is H or CH<sub>3</sub> and R<sup>1</sup> is H or CH<sub>3</sub>.

5

20. The compound according to claim 17, wherein said linking group is a carbonyl group of the formula:



wherein said carbonyl group represents an end-terminal carboxyl group of said polymer to which S is covalently joined by an ester linkage.

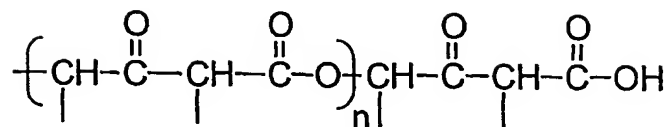
10

21. The compound according to claim 17, wherein said biodegradable polymer is selected from the group consisting of poly alpha-hydroxy acids, polyanhydrides, polyorthoesters, polyacrylates, polycaprolactones, polycarbonates, polyfumarates, and copolymers thereof.

15

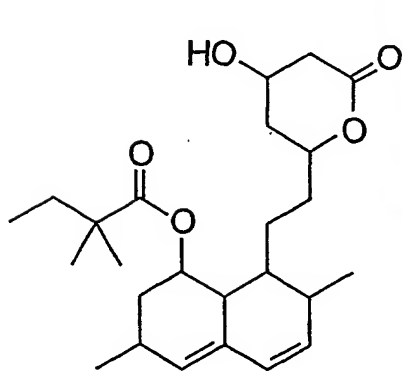
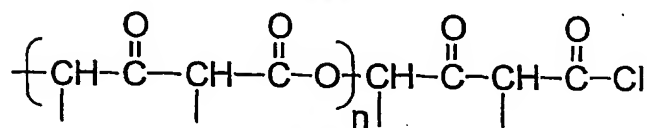
22. The compound according to claim 17, wherein said biodegradable polymer is a polyester polymer selected from the group consisting of poly(lactic acid), poly(glycolic acid), poly(caprolactone), and copolymers thereof.

20

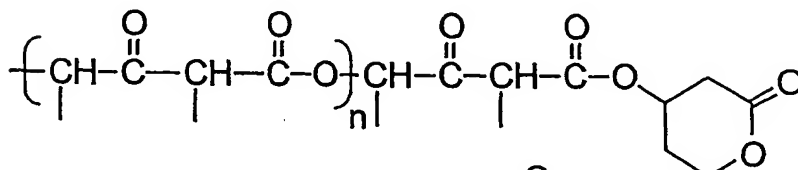
FIG. 1

POLYLACTIDE, INHERENT VISCOSITY~132,000

$\begin{array}{c} \text{COCl} \\ | \\ \text{COCl} \end{array}$ 
 $\begin{array}{c} \text{OXALYL} \\ \text{CHLORIDE} \end{array}$



SIMVASTATIN

PYRIDINE/CH<sub>2</sub>Cl<sub>2</sub>

PLG-STAT

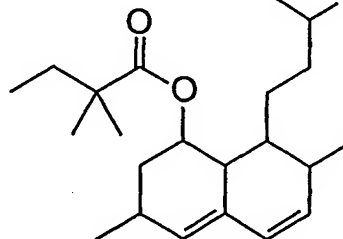




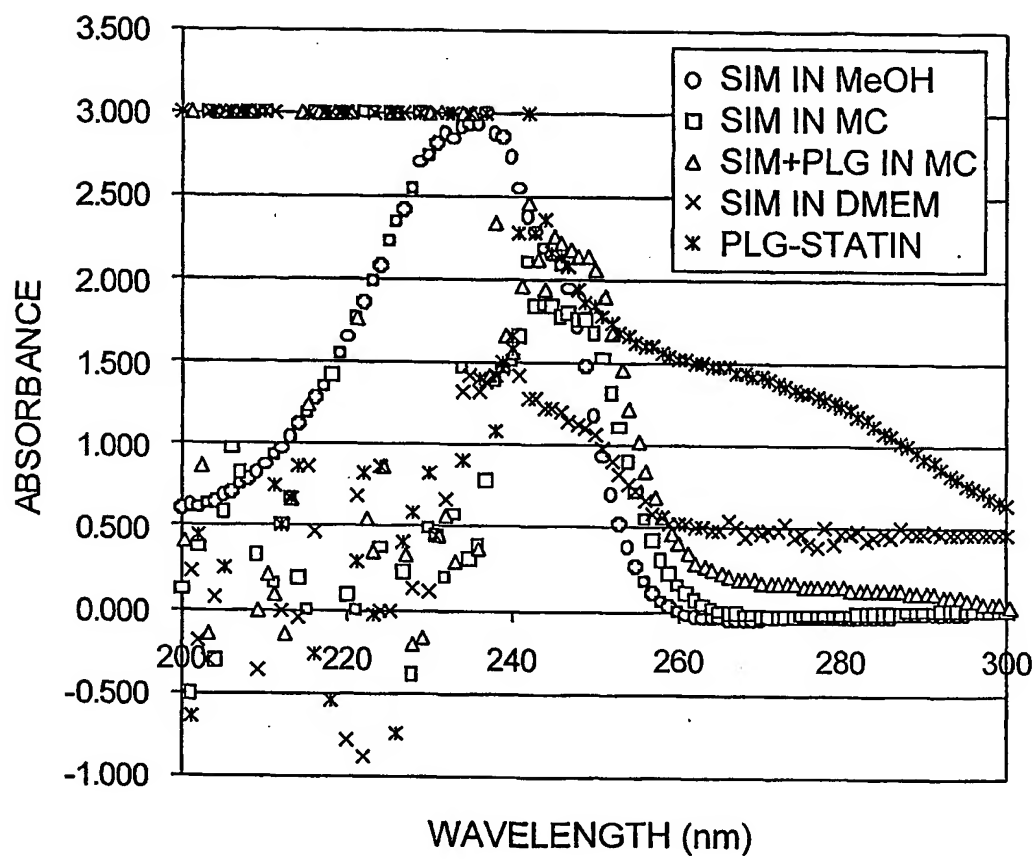
FIG. 2

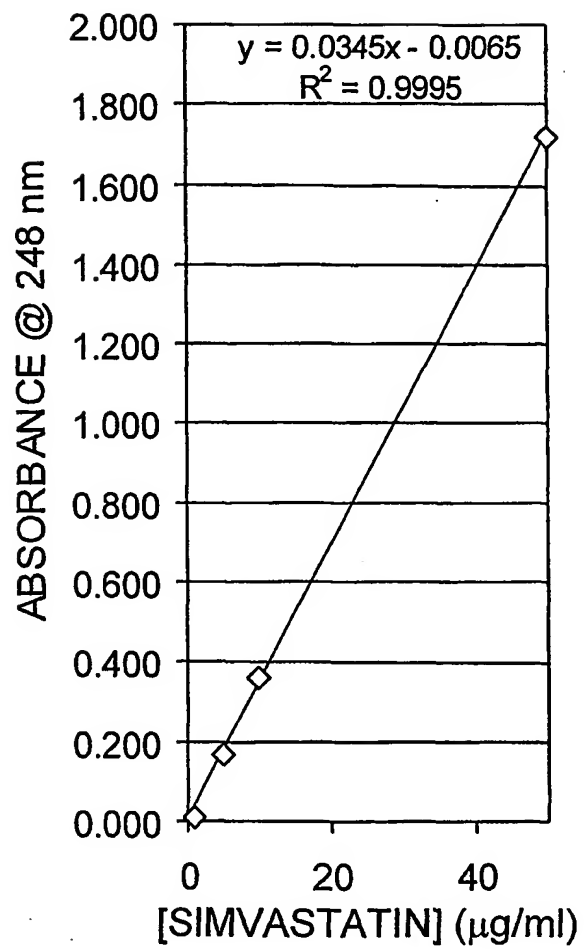
FIG. 3

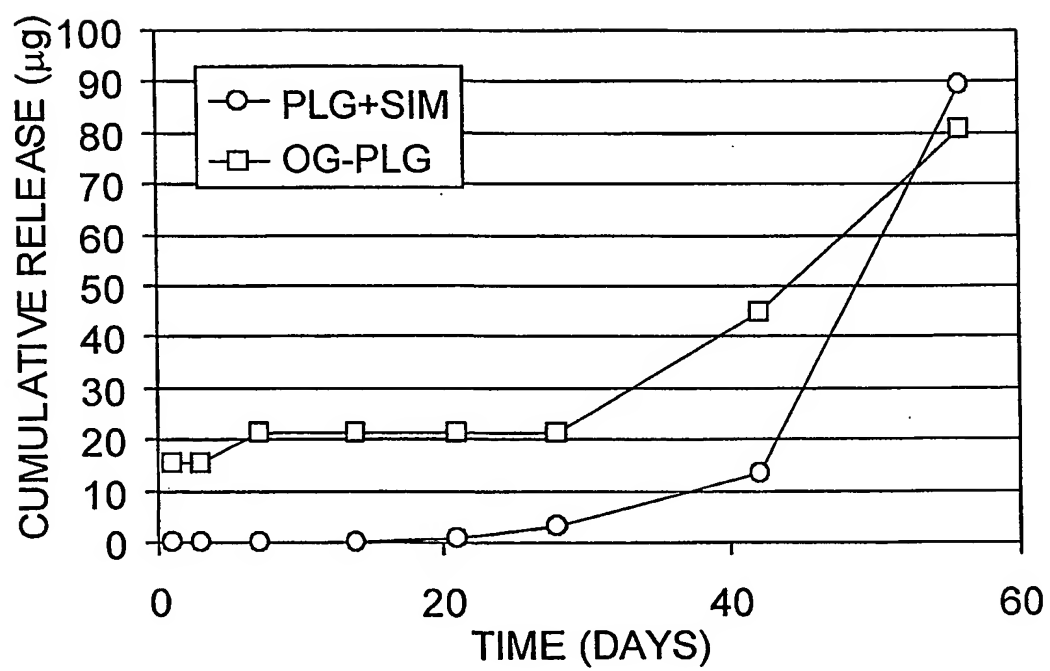
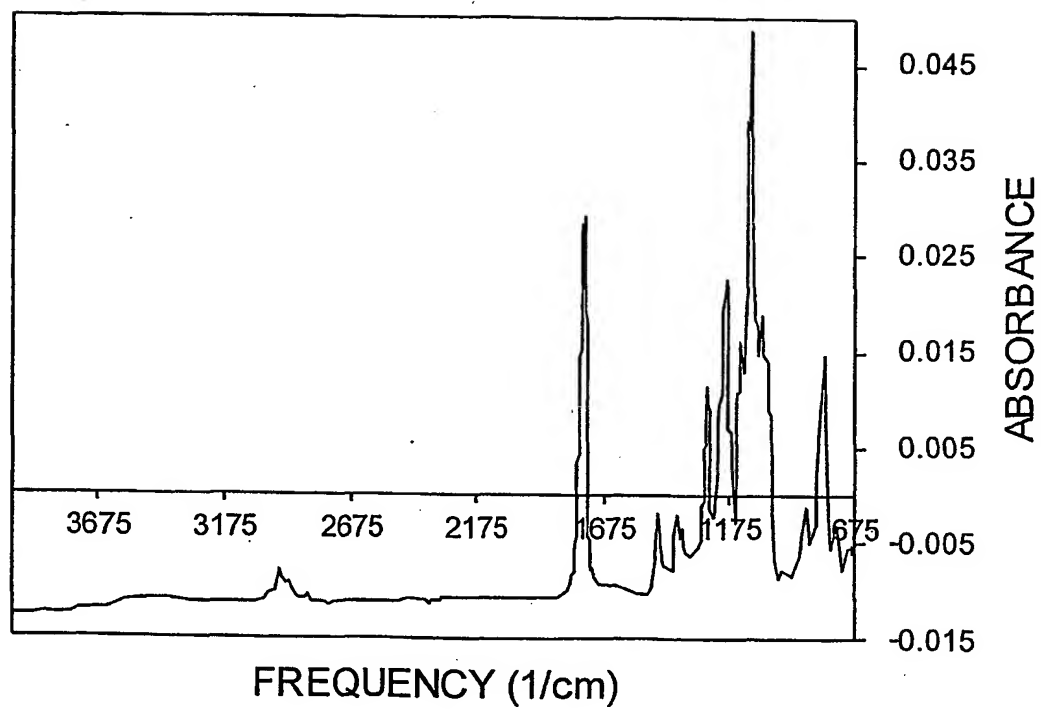
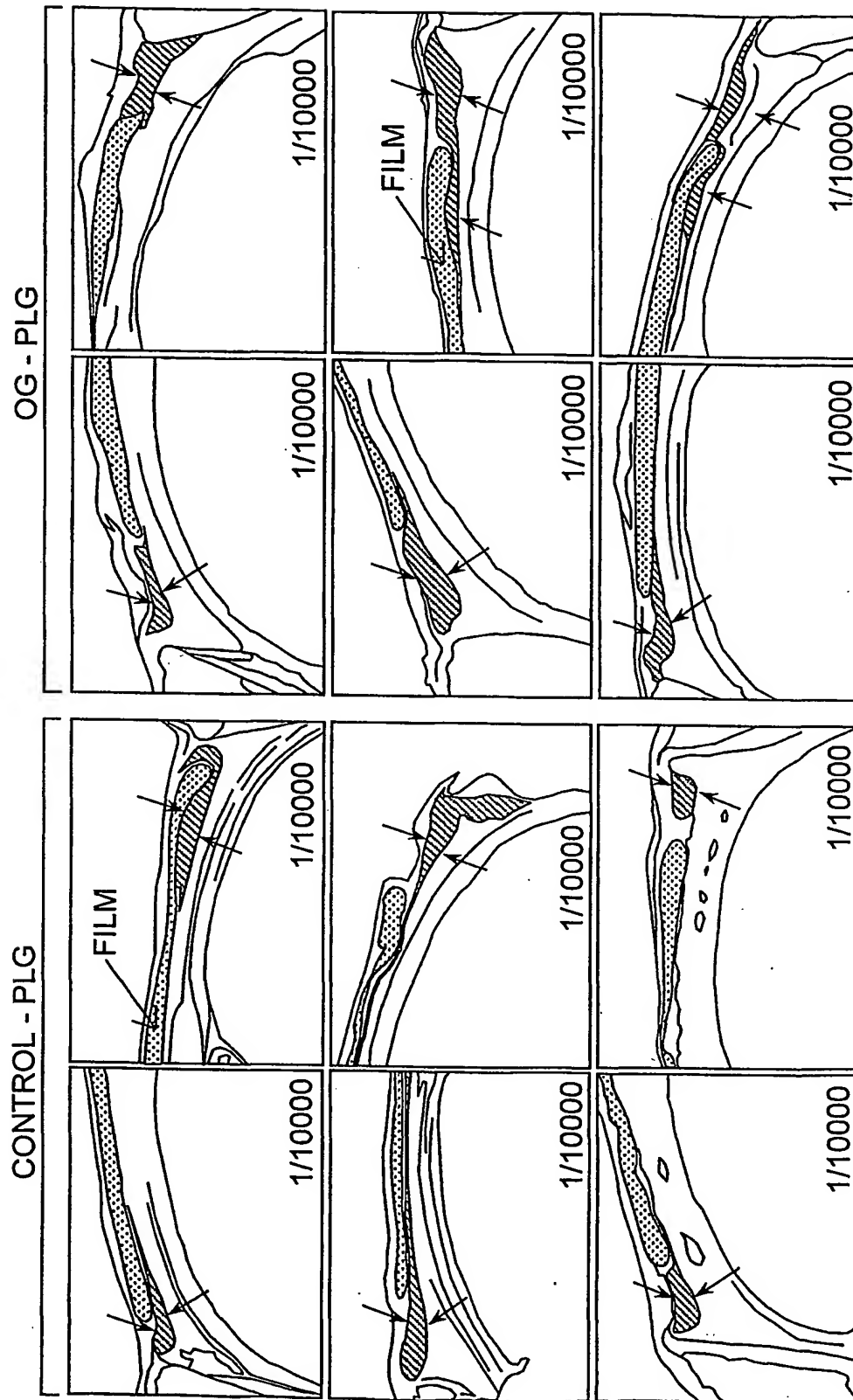
FIG. 4

FIG. 5



**FIG. 6**

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
24 October 2002 (24.10.2002)

PCT

(10) International Publication Number  
**WO 2002/082973 A3**

- (51) International Patent Classification<sup>7</sup>: C12N 5/00, A61K 31/225
- (21) International Application Number: PCT/US2002/011519
- (22) International Filing Date: 15 April 2002 (15.04.2002)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 60/284,107 16 April 2001 (16.04.2001) US
- (71) Applicant (*for all designated States except US*): BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM [US/US]; 201 West Seventh Street, Austin, TX 78701 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): WHANG, Kyumin [KR/US]; 14027 Cougar Rock Dr., San Antonio, TX 78230 (US). SATSANGI, Neera [US/US]; 12823 Hunting Bear, San Antonio, TX 78249 (US). NORLING, Barry, K. [US/US]; 13427 Shorecliff St., San Antonio, TX 78248-1213 (US).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:  
— with international search report
- (88) Date of publication of the international search report: 11 March 2004
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: OSTEOTROPIC BIOMATERIALS, METHODS OF USE THEREOF AND IMPLANT SYSTEMS INCORPORATING THE SAME

(57) Abstract: An osteotropic compound of the Formula P-L-S is described, wherein P is a biodegradable polymer such as a polyester polymer, L is a linking group such as a covalent bond or carbonyl, and S is an osteotropic group such as a statin. Scaffold bodies formed from such compounds are described, along with such bodies containing bone cells such as bone marrow cells and methods of use thereof.



WO 2002/082973 A3

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/11519

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 5/00; A61K 31/225

US CL : 424/93.7; 514/547

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

..minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.7; 514/547

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Continuation Sheet

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,990,194 A (DUNN et al.) 23 November 1999 (23.11.99), see the entire document.	1-22

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

30 September 2002 (30.09.2002)

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703)305-3230

Date of mailing of the international search report

12 MAR 2003

Authorized officer

Jean C. Witz

Telephone No. (703) 308-0196

# INTERNATIONAL SEARCH REPORT

PCT/US02/11519

Continuation of B. FIELDS SEARCHED Item 3:  
CAPLUS, INPADOC, MEDLINE, BIOSIS, EAST  
search terms: HMG Co-A, bone cells



**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

☒ **BLACK BORDERS**

☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**

☐ **FADED TEXT OR DRAWING**

☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**

☐ **SKEWED/SLANTED IMAGES**

☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**

☐ **GRAY SCALE DOCUMENTS**

☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**

☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**

☐ **OTHER:** \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**